

ABSTRACT OF THE DISCLOSURE

A fluorescence observation apparatus includes a light source, an illumination optical system conducting irradiation light from the light source to a specimen, an aperture member provided in the illumination optical system, a first wavelength selective member, a light splitter deflecting the irradiation light to conduct the light to the specimen, an objective lens interposed between the light splitter and the specimen, a second wavelength selective member transmitting fluorescent light emanating from the specimen, a detecting device receiving the fluorescent light, and a projection optical system projecting the aperture member at the pupil position of the objective lens. In this case, the aperture member has a partial aperture through which part of the irradiation light passes, and the size of the partial aperture and the magnification of the projection optical system are set to satisfy the following Conditions:

$$0.5NA \leq NA_1 < NA$$

$$NA_1 < n$$

where NA_1 is a numerical aperture derived from an angle made by a ray closest to the optical axis, of rays of light passing through the partial aperture, with the optical axis on the specimen, NA is the maximum numerical aperture of the objective lens, and n is the refractive index of a medium holding the specimen.